PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Daniel JAVITT

Docket No: A8311

Appln. No.: 10/066,657

Group Art Unit: 1617

Confirmation No.: 5724

Examiner: Theodore J. Criares

Filed: February 06, 2002

For: D-SERINE TRANSPORT ANTAGONIST FOR TREATING PSYCHOSIS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Daniel Javitt, hereby declare and state:

THAT I am a citizen of the United States of America;

THAT I have received an M.D. Degree in 1983 and a Ph.D. Degree in 1990 from the Albert Einstein College of Medicine.

THAT I have been employed by the Nathan Kline Institute since 1997, where I hold a position as Psyhiatrist III (research), with responsibility for Directorship of Program in Cognitive Neuroscience and Schizophrenia; and

THAT I am the inventor of the subject matter of the above-identified application and I am familiar with the proceedings in the prosecution of the present application.

I provide the following as evidence that the claims are enabled by the specification as originally filed and in support of the novelty and nonobviousness of the claimed invention in view of the prior art.

D-serine transport inhibitors as new treatments for schizophrenia;
Preclinical Characterization – August 16, 2004

Rationale

Schizophrenia is associated with disturbances in neurotransmission at *N*-methyl-D-aspartate (NMDA) type glutamate receptors that may be ameliorated by the administration of agents that augment NMDA receptor-medicated neurotransmission by stimulating the NMDA-associated glycine binding site. Endogenous ligands for this site include glycine and D-serine. Administration of these agents has been shown to effectively ameliorate persistent negative and cognitive symptoms of schizophrenia, supporting the underlying hypothesis. However, required doses of these agents are high, and D-serine has been associated with nephrotoxicity in rodents although not, to date, in primates. More refined approaches to stimulating the glycine/D-serine binding site of the NMDA receptor, therefore, may be desirable.

Glycine levels in brain are regulated by well described transport systems, including GLYT1 and SNAT2, which serve to maintain low glycine levels in the immediate vicinity of the NMDA receptor complex. Thus, suggesting that inhibition of these transport systems might lead to enhanced NMDA receptor-mediated neurotransmission. Preclinical data with prototypic glycine transport inhibitors, including GDA (1, 2), NFPS (3-5) and Org 25498 (6), as well as clinical findings with the non-selective glycine transport inhibitor sarcosine (7), support the role of glycine transport processes in NMDA regulation and schizophrenia treatment.

As compared with glycine transport processes, D-serine transport processes are relatively poorly described. As preliminary data for this project, we demonstrated the existence of a high-affinity, selective D-serine transport process in rat brain synaptosomes (8). Further, several

neuronally derived cell lines were identified that expressed endogenous D-serine transport, permitting their use in the screening of potential high affinity D-serine transport inhibitors that could then be used to evaluate functional importance of D-serine transporters in regulating processes of relevance to schizophrenia. This report describes initial preclinical results with 3 lead compounds determined from a screening library of 200+ D-serine derivative manufactured under contract by Albany Molecular Research, Albany, NY.

Compound Description

Initial structures were determined by analogy to high affinity inhibitors for GLYT1 and other amino acid transporters. The D-serine backbone was chosen as the starting point from synthesis. Hydrophobic derivative compounds were made by substation at the amino, carboxyl and alcohol positions of the D-serine molecule. Screening was done recursively, with substitutions producing a higher potency blockade which was retained in subsequent rounds of synthesis. The initial goal of this project was identification of compounds with $<10^{-5}$ M (100 μM) affinity for the D-serine transport process expressed either in cell culture or cortical synaptosomes. Three compounds were identified with requisite potencies, which were also amenable to scale up synthesis to quantities needed for *in vivo* investigation. Affinities of these compounds for inhibition of D-serine transport are shown in Table 1. Binding curves are shown

Table 1: IC_{50} values for inhibition of D-SERINE transport by prototype D-SERINE transport inhibitors: human neuroblastoma cells and cortical synaptosomes

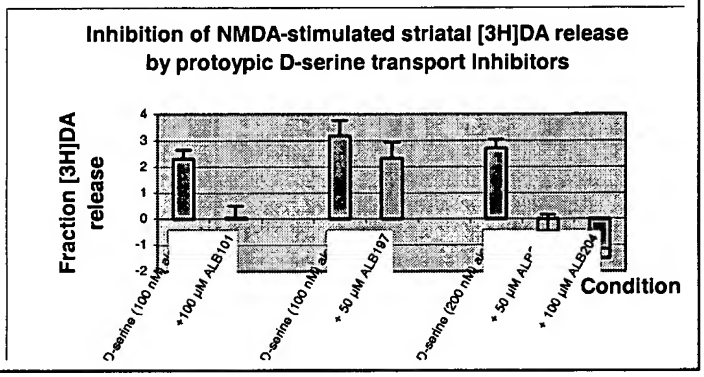
	cell culture (neuroblastoma)	Rat cortical synaptosomes
ALB101	16.3 μM (range: 3.46 - 77.1)	83.0 μM (range: 19.8 – 3485)
ALB197	7.3 μM (range: 2.2 – 23.9)	44.5 μM (range: 8.2 – 240.6)
ALB204	20.2 μM (range: 4.5 – 89.2)	264 μM (range: .017 – 4046)

in Appendix A. All compounds showed <50% inhibition of transport mediated via other neural transport systems at concentrations that substantially abolished high-affinity D-serine transport.

In vitro Dopamine Release

Initial screening was performed using an *in vitro* screening system that has previously been shown to be sensitive to effects of NMDA augmenting agents, including glycine and glycine transport inhibitors (9, 10). In this system, isolated mouse striatum is preincubated with [^{3}H]DA, which is incorporated into presynaptic dopaminergic stores. DA release is then initiated by brief exposure to NMDA. The effect of test agents on NMDA-stimulated DA release is measured. Schizophrenia, in general, is associated with hyperactivity of striatal DA release (11). A common action of glycine and glycine transport inhibitors, therefore, is inhibition of NMDA stimulated DA release (9, 10). The three lead compounds (ALB101, ALB197 and ALB204) were therefore screened for their ability to inhibit release. Assays were conducted in both the absence and presence of added D-serine. No statistical difference was found between the two conditions, although results appeared more consistent in the presence of low concentrations of D-serine (100-200 nM), potentially recreating the endogenous *in vivo* situation. All 3 lead compounds produced effects in the expected direction (Figure 1), with results being most robust for ALB101 (t=3.59, df=34, p<.0004) and ALB204 (50uM: t-4.84, df=28, p<.0001; 100 μM:

Figure 1: Effect of prototype D-SERINE transport inhibitors on NMDA stimulated DA release



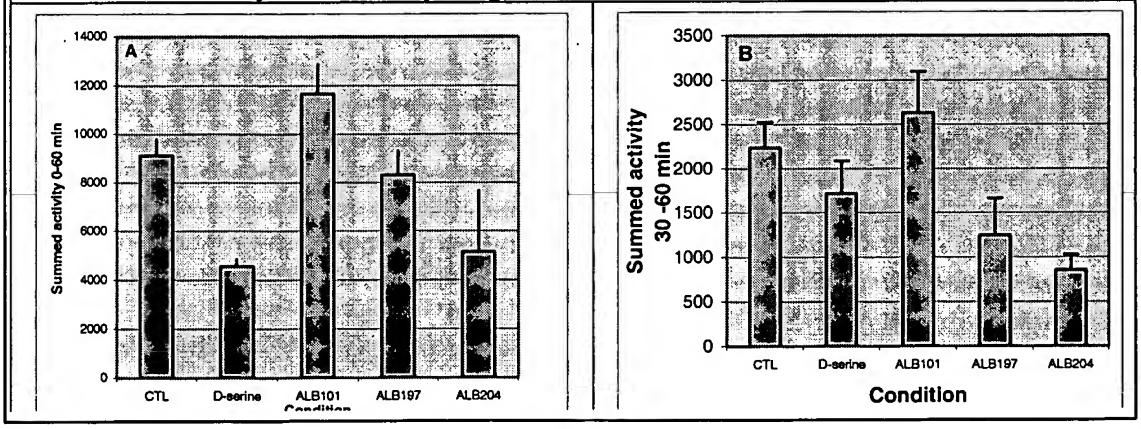
t=8.61, df=33, p<.0001). Mean response curves from the release assay experiment are shown in Appendix B.

In vivo Behavior

Finally, in order to assess relative *in vivo* potency of prototypic glycine transport inhibitors, locomotor hyperactivity was evaluated in response to amphetamine or PCP challenge. In prior studies, we demonstrated that glycine and the prototypic glycine transport inhibitor GDA inhibited hyperactivity induced by PCP but not amphetamine (1), with similar reports being observed as well for the high affinity glycine transport inhibitors NFPS and Org 24461 (12). In these studies, effects of D-serine were compared to those of the prototype compounds ALB101, ALB197 and ALB204.

In our preliminary studies, a somewhat different profile of activity was observed for D-serine, with D-serine treatment leading to inhibition of both amphetamine- (Fig. 3A) and PCP (Fig. 3B)-induced hyperactivity at a dose of 25 µmol/g (=2625 mg/kg). Of these, only the effect

Table 3: Effect of D-SERINE and prototype D-SERINE transport inhibitors on locomotor activity induced by amphetamine (*A*, left) and PCP (*B*, right)

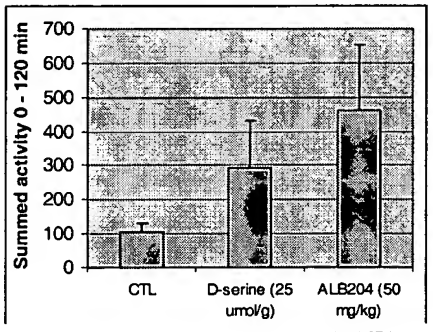


on amphetamine-induced hyperactivity proved statistically significant (t=5.54, df=107, p<.0001). The need for high doses of D-serine to reverse hyperactivity induced by NMDA antagonists is

consistent with previous reports in this area. In particular, Nilsson et al. (13) observed significant inhibition of MK-801-induced hyperactivity only at a dose of 4000 mg/kg. Significant effects in the expected direction were observed for prototypic D-serine transport inhibitors as a group. Results were again most significant for ALB204, which produced significant inhibition of both amphetamine (t=3.23, df=81, p=.002) and PCP (t=2.61, df=49, p=.012) induced hyperactivity at a dose of 100 mg/kg ip. This compound was thus approximately 25-fold more effective than D-serine in producing its behavioral effects. Activity curves are shown in Appendix C.

For initial studies with these compounds, Balb/c mice were used for PCP-induced hyperactivity studies because these mice are known to show greater locomotor hyperactivity to PCP than the more commonly used C57 mice (14). In order to verify, however, that effects of D-serine and ALB204 were not due to nonspecific locomotor depressant effects, studies with ALB204 were performed also in C57 mice. In these mice, we previously observed (unpublished observations) that D-serine produces a significant enhancement of locomotor activity following PCP administration, presumably reflecting reversal of PCP-induced hypoactivity similar to that observed in primates. In C57, mice, as we observed previously, D-serine produced increased locomotor activity (distance traveled) that was significant at trend level (t=1.79, df=21, p=.087). In contrast, ALB204 produced a robust increase in activity that was statistically significant (t=2.65, df=20, p=.016), and in the opposite direction from its effects in Balb/c mice. Thus, anti-PCP effects of ALB204

Figure 4: Effect of D-SERINE and ALB204 on PCP-induced activity – C57 mice



cannot be attributed to general locomotor depressant effects, and thus may be mediated by significant potentiation of NMDA receptor-mediated neurotransmission.

Conclusion

The present studies were performed with the goal of exploring feasibility of developing D-serine transport inhibitors as potential treatments for schizophrenia. The results of the parallel *in vitro* and *in vivo* investigations demonstrate first the "druggability" of the D-serine transport inhibitor site, with compounds from a screening library showing predictable inhibitory effects on D-serine transport; and second, that compounds targeting the D-serine transport site show predicted effects in models previously shown to be sensitive to effects of NMDA receptor agonists and antagonists. Of the compounds screened, ALB204 showed greatest *in vitro* and *in vivo* effectiveness and may be an appropriate compound for future, large scale drug development studies.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: _____

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Dr. Daniel Javitt

References

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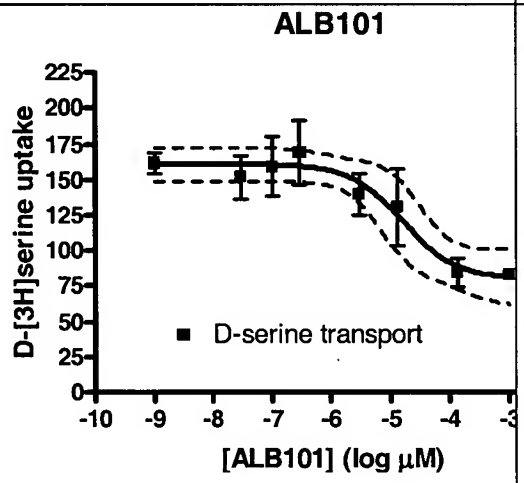
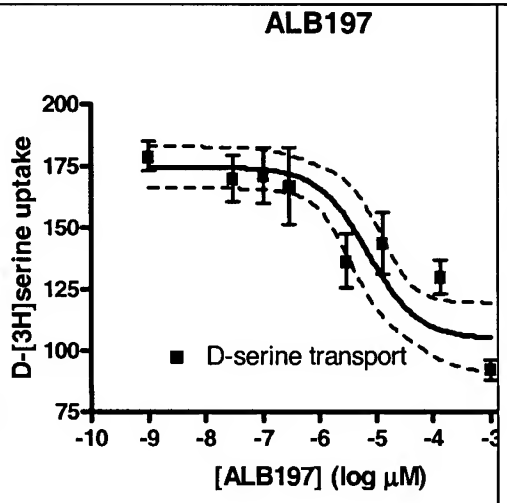
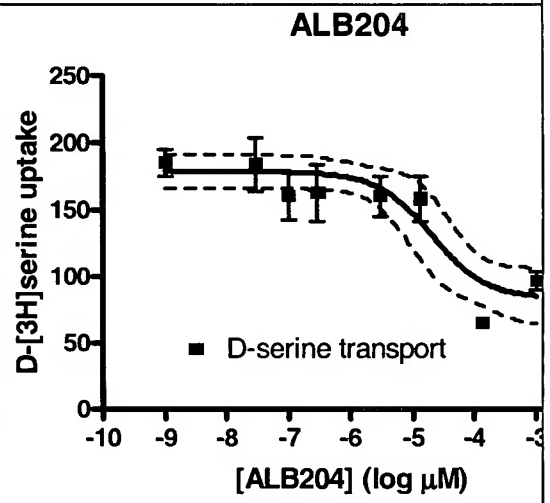
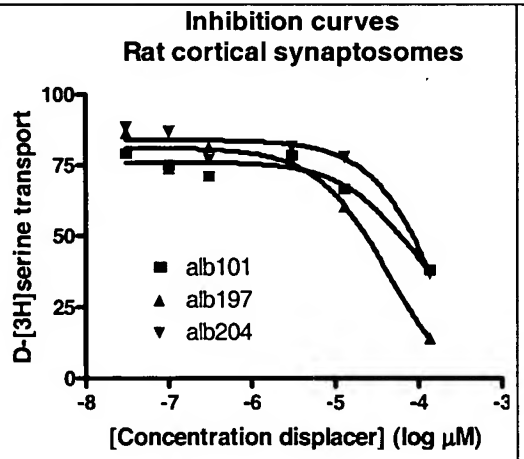
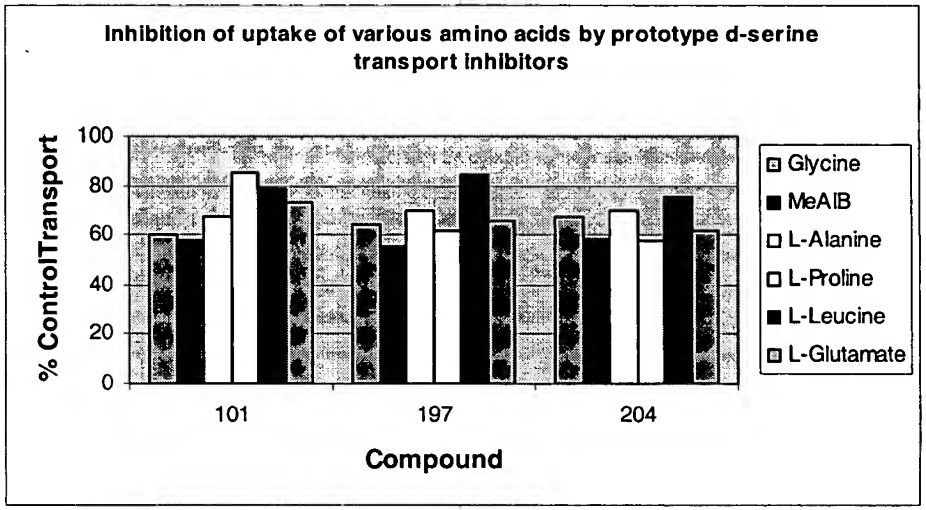
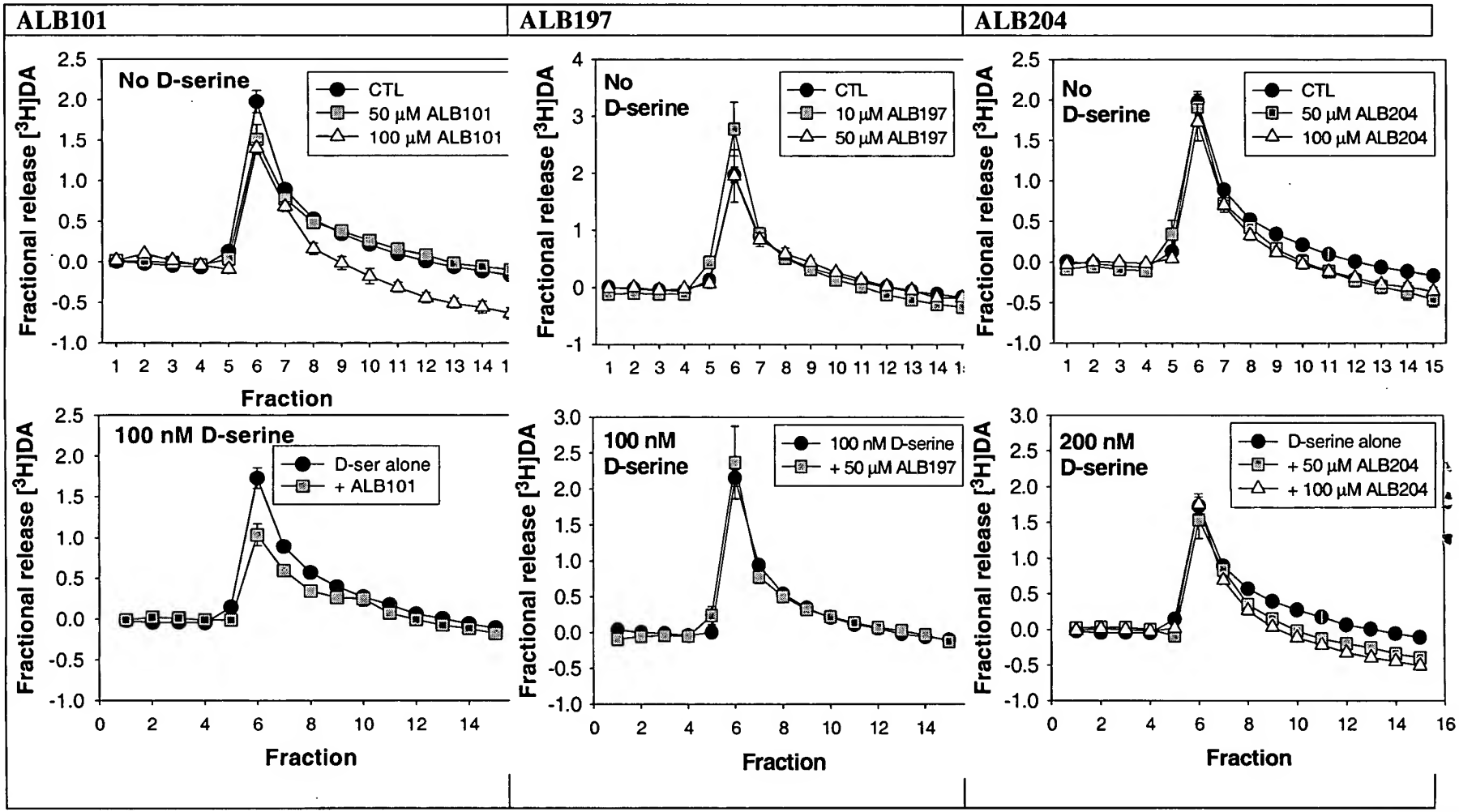
Appendix A: Summary of transport studies with prototypic D-serine transport inhibitors

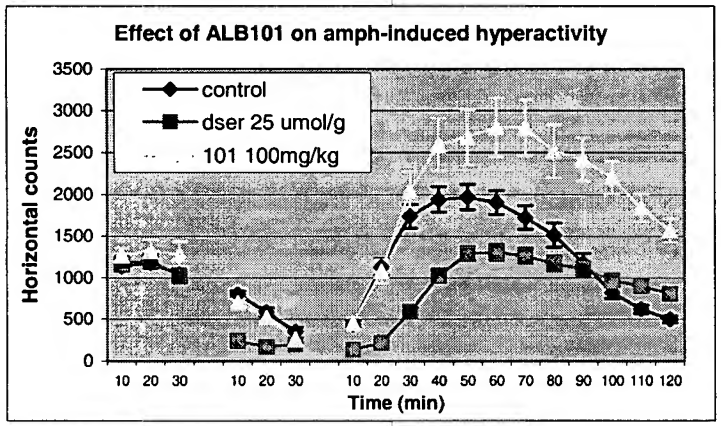
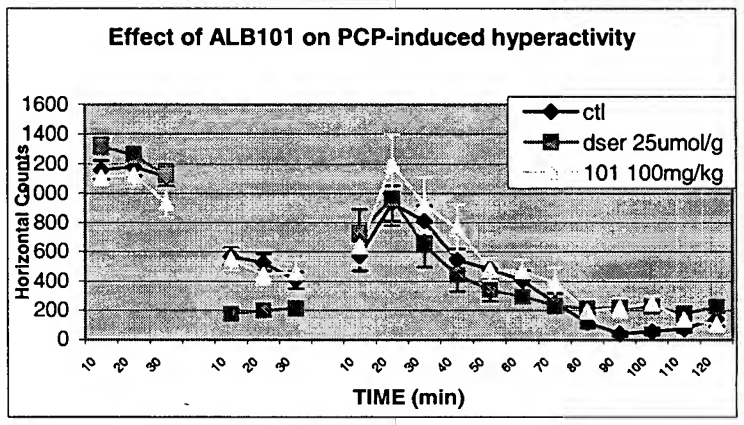
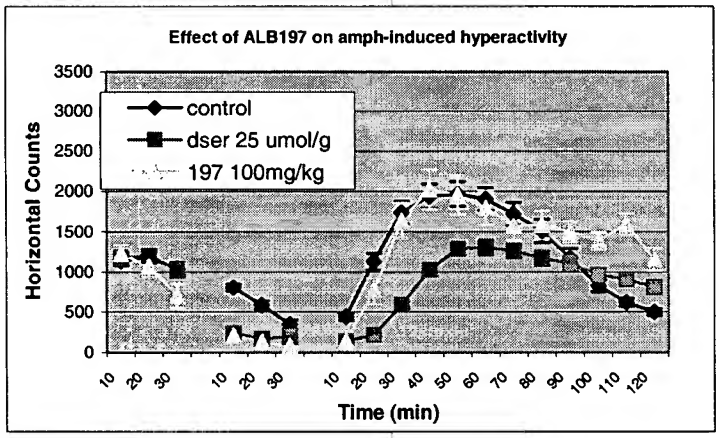
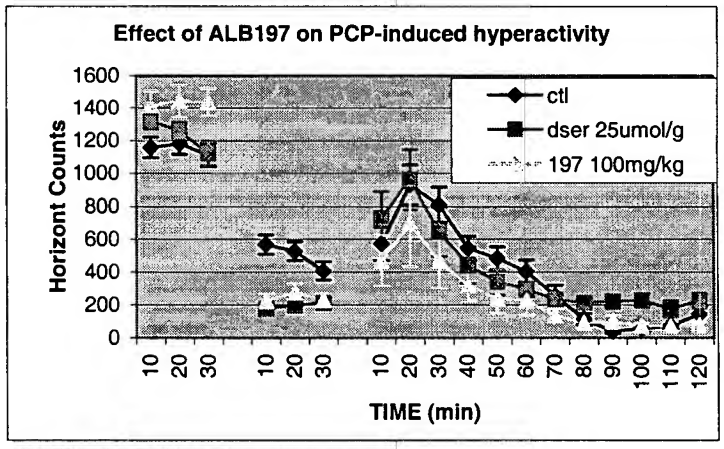
cell culture (2273 cells)			

rat cortical synaptosomes		

Appendix B: Effect of Prototype D-serine **transport inhibitors on NMDA (300 µM)-induced DA release from isolated mouse striatum *in vitro***



Appendix C: Summary of animal behavior (locomotor hyperactivity) studies

	Amphetamine-induced hyperactivity	PCP-induced hyperactivity
101		
197		

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